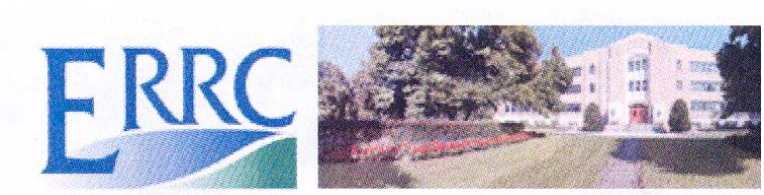

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Title: Irradiation and Modified Atmosphere Packaging of Endive Influences Survival and Regrowth of *Listeria monocytogenes* and Product Sensory Qualities

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Irradiation and modified atmosphere packaging of endive influences survival and regrowth of *Listeria monocytogenes* and product sensory qualities ☆

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Abstract

Cut pieces of endive were inoculated with *Listeria monocytogenes*, packaged in gas-impermeable bags in air, 5/5/90% or 10/10/80% CO_2, O_2 and N2 ("Air-0", "5/5" and "10/10", respectively) and irradiated to 0.0 (control), 0.3 or 0.6 kGy. At various times during refrigerated storage, samples were taken and a determination made of (a) total microflora, (b) *L. monocytogenes*, (c) headspace gas composition, (d) color and (e) texture. Irradiation reduced initial microbial counts in a dose-dependant manner. Bacteria regrew during storage on Air-0 samples, but not on 5/5 or 10/10 samples. In each of the three atmospheres, O_2 declined and CO_2 increased, irrespective of radiation dose. Irradiated leaf material in Air-0 tended to retain color attributes during storage better than non-irradiated; color retention was more variable under 5/5 and 10/10 packaging. After 8 days, maximum shear force relative to the initial level was significantly reduced in 5/5 at all radiation doses, was not significantly changed in Air-0, and was dose-dependent in 10/10. By 14 days, the texture of all samples had degraded significantly. These results indicate that irradiation and modified atmosphere packaging can be combined to prevent the regrowth of *L. monocytogenes* during post-irradiation refrigerated storage, thereby improving product safety.

Keywords: Irradiation; Vegetable; Foodborne pathogen; Storage

1. Introduction

Fresh vegetables have been implicated in numerous recent outbreaks of foodborne illness (Beuchat, 1996; Haldane, 2003). Salad vegetables, including fresh-cut lettuce, can be a source of pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* and *Shigella* spp. (Gombas et al., 2003; Horby et al., 2003; Tauxe et al., 1997). Ionizing radiation can effectively eliminate human pathogens from leafy salad vegetables such as lettuce (Foley et al., 2002; Niemira et al., 2002) and endive (Niemira et al., 2003). Studies with *L. monocytogenes*-inoculated vegetables have shown that an initial reduction in the pathogen population resulting from a low irradiation dose (sufficient to reduce the bacterial population by approximately 2 $\log_{10}$ units) can be followed by a regrowth in refrigerated storage; higher doses (equivalent to reductions of approximately 4 $\log_{10}$ units) result in a more persistent suppression (Prakash et al., 2000b; Niemira et al., 2003).

☆ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Modified atmosphere packaging (MAP) is used commercially to suppress the growth of spoilage organisms and extend the shelf life of vegetable and meat products (Yuan, 2003). MAP may be passive, in which packages are sealed in air, or active, in which a defined mixture of gases are used to flush the package, typically with reduced O_2 and increased CO_2, with the balance composed of N_2. For vegetables packaged under either system, there is no single ideal or standard gas mixture; the mixture of gases within the package changes over time in response to the respiration of the produce and the gas permeability of the packaging material, and the specific vegetable under consideration (Al-Ati and Hotchkiss, 2002).

The potential for combining MAP with low-dose irradiation has been explored in a variety of foods, including lettuce (Hagenmaier and Baker, 1997; Prakash et al., 2000a). However, the extent to which headspace gas composition influences the regrowth of irradiated *L. monocytogenes* on vegetables is poorly understood, particularly with regard to the bacteriostatic effects of elevated CO_2 levels on spoilage and pathogenic bacteria (Yuan, 2003). The behavior of vegetable-associated pathogens after irradiation treatment will help to determine the applicability of irradiation to these products as an antimicrobial intervention. The objectives of this study are to determine the effects of irradiation and refrigerated storage on the survival and regrowth of *L. monocytogenes* on endive leaves packaged under three different modified atmospheres, and the sensory quality of endive leaves so treated and stored.

2. Materials and methods

Whole heads of endive were purchased from local markets less than 24 h prior to the start of each experiment. The outer leaves were discarded, and cut leaf pieces were prepared from the entire head. The basal portion of the head was removed, approximately 5 cm from the end. The leaves were sliced as a group into pieces weighing approximately 0.5 g. Fresh produce typically carries a native microbial load (Niemira, 2003); before use in the experiments, the leaf material was surface sanitized with a 300 ppm sodium hypochlorite solution. The leaf pieces were gently agitated in the sanitizing solution at room temperature for 3 min, thoroughly rinsed in distilled water, and spun in a sterile salad spinner-type centrifuge (Oxo International, New York, NY) to remove excess surface water. The microflora of sanitized leaf material was sampled with a surface wash with Butterfield's phosphate buffer (BPB, Applied Research Institute, Newtown, CT), serial dilution, pour plating with tryptic soy agar (TSA, Difco, Detroit, MI) and incubation at 37°C for 24 h. The post-sanitization population was found to be less than 20 cfu/g leaf tissue.

2.1. Strains and preparation of media

Two strains of *L. monocytogenes* (ATCC 49594 and ATCC 43256, American Type Culture Collection, Manasass, VA) were maintained on 50% glycerol at −70°C. A frozen culture was sampled and regrown in tryptic soy broth (TSB, Difco, Detroit, MI) for 16 h at 37°C with agitation and streaked onto Palcam agar (Difco, Detroit, MI). This was incubated at 37°C for 48 h to form single colonies. These colonies were used to inoculate fresh TSB, grown for 16 h at 37°C with agitation, for each experiment. The cell density of the starting inoculum was determined by serial dilution with sterile BPB and pour plating with TSA. The cell density was typically 10^9 cfu/ml. The starting inoculum was diluted 1:10 with sterile BPB to make a working inoculum.

2.1.1. Inoculation of leaf pieces

Cut leaf pieces were inoculated separately as described previously (Niemira et al., 2003). Briefly, sanitized leaf pieces were transferred to a sterile bin and 2000 ml of the working inoculum was added. The material was agitated gently for 120 s to completely submerge each piece, and then transferred to a sterile salad spinner-type centrifuge (Oxo International, New York, NY). The material was spun twice to remove excess inoculum from the surface of the leaf pieces.

2.2. Modified atmosphere packaging

In order to ensure that the material would experience a relatively high-CO_2, low-O_2 environment, samples (45 g) were placed in a laminated foil/plastic barrier-type bag (MIL-B-131, Bell Fibre Products, Columbus, GA). Bagged samples were placed in a self-contained gas packer/sealer (model #A300/16 MC, Multivac Inc., Kansas City, MO). The bags were flushed with air (passive MAP, "Air-0"), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 (5/5) or 10% CO_2, 10% O_2, 80% N_2 (10/10) (Scott Specialty Gases, Plumsteadville, PA). In separate experiments for sensory analyses, non-inoculated samples of endive leaves were bagged in the three atmospheres as described. The ratio of headspace gas to leaf pieces was approximately 10:1 (v/v). The samples were refrigerated (4°C) until irradiation, typically 30–60 min.

2.3. Irradiation and storage

The samples were irradiated using a Lockheed-Georgia (Marietta, GA) cesium-137 temperature-controlled gamma radiation source, with a dose rate of

5.88 kGy/h, according to the method of Niemira et al. (2003). The bagged samples were given doses of 0.0 (control), 0.3 or 0.6 kGy, and temperature was held at 4°C during irradiation. Alanine pellets (Bruker, Inc. Billerica, MA) were used for dosimetry. The pellets were read on a Bruker EMS 104 EPR analyzer and compared with a previously determined standard curve. The delivered dose, as determined by EPR dosimetry, was typically within 5% of the nominal dose.

Following irradiation, the bagged samples were stored at 4°C. In the experiments related to headspace gas and microbiology, samples were evaluated 60-90 min after irradiation (day 0), and after 1, 2, 5, 9, 14 and 19 days in refrigerated storage. In the experiments related to sensory properties, samples were evaluated after 1, 8 and 14 days in refrigerated storage. The samples consisted of one bag per dose/time/atmosphere combination. The experiment was performed three times.

2.4. Gas sampling

At each of the sampling times, the sample bags were inspected for leaks. A 0.5 ml aliquot of the headspace gas in the sample bag was taken using a syringe and a fine gauge needle, pierced through the bag material. The sampling hole was sealed with electrical tape. The gas samples were analyzed with a Gow-Mac series 580 gas chromatograph (Gow-Mac Instrument, Bridgewater, NJ), equipped with a 183 cm CTR I column (Alltech Associates, Inc., Deerfield, IL) and a thermal conductivity detector. The injector, oven and detector temperatures were held at ambient (23–25°C). The carrier gas was helium with a flow rate of 120 ml/min. CO_2 and O_2 levels were calculated in comparison to a standard (Alltech Associated, Deerfield, IL). After the headspace gas sampling was completed, the bagged samples were set aside for microbiological sampling.

2.5. Microbiological sampling

Sterile BPB was added to each of the sample bags (180 ml). The bags were closed and agitated for 2 min to obtain a surface wash of the leaf pieces. A 1 ml aliquot was taken, and serially diluted with sterile BPB. The diluted sample was pour plated with Palcam agar to enumerate the population of *L. monocytogenes*, and with TSA to enumerate the total microbial population (inoculated *L. monocytogenes* plus remaining background microflora). Three plates per dilution were made in each medium. The plates were allowed to solidify and incubated at 37°C for 48 h, and counted with an automated plate counter (AccuCount 1000, BioLogics, Gainsville, VA), and scaled to cfu/g leaf tissue.

2.6. Sensory properties

2.6.1. Texture

The maximum shear strength of the leaf pieces was measured with a TA.XT2i texture analyzer running the Texture Expert v.1.22 software package (Texture Technologies, Scarsdale, NY) using a TA-91 Kramer Shear Press with five blades.

2.6.2. Color

At each sampling time, color values were taken with a Hunter Lab Miniscan XE meter (Hunter Laboratory, Inc., Reston, VA) to determine the brightness (*L*-value), greenness/redness (*a*-value) and blueness/yellowness (*b*-value) of the leaf material. The meter was calibrated using white and black standard tiles. Illuminant D65, 10° standard observer, and a 2.5 cm port/viewing area were used.

2.7. Statistics and data analysis

All microbiological, texture, color and headspace gas composition data were subjected to analysis of variance (ANOVA, SigmaStat, SPSS, Chicago, IL) to evaluate the effect of irradiation dose at each sampling time, the effect of time in storage, and the effect of gas mixture. Regrowth of *L. monocytogenes* after irradiation has been previously shown as an increase approaching a maximum population level as a function of time in storage (Niemira et al., 2003). In this study, regrowth of *L. monocytogenes* and total microbiological populations were modelled for each of the nine gas/dose combinations using a hyperbolic function of the form $y = y_0 + ax/(b + x)$ (SigmaPlot, SPSS, Chicago, IL). In this function, y is the population ($\log_{10}$ cfu/g), x is time (days), y_0 is the population immediately post-irradiation on day 0, and the population growth curve, approaching an asymptotic maximum, is determined by the parameters a and b.

3. Results and discussion

3.1. Microbiological reduction and regrowth

The recoverable counts following inoculation (day 0) were approximately 6.5 $\log_{10}$ cfu/g leaf material. After 2 days in storage, the non-irradiated Air-0 samples showed significantly ($P<0.05$) increased counts of *L. monocytogenes* (Fig. 1A) and total microflora (Fig. 2A), rising from the relatively high initial level of associated bacteria immediately following inoculation, 6.2–6.8 $\log_{10}$ cfu/g and remaining consistent for the remainder of the study. In contrast, on non-irradiated 5/5 and 10/10 samples the populations of *L. monocytogenes* (Fig. 1B

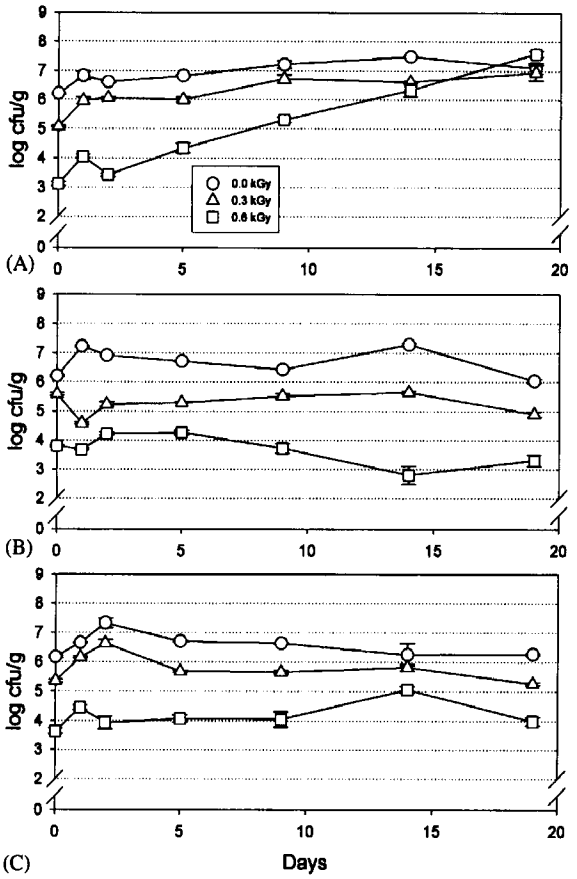

Fig. 1. Populations of *L. monocytogenes* from packaged, irradiated endive, plated on Palcam agar. Packaging atmospheres are air ("Air-0", graph 1A), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 ("5/5", graph 1B) or 10% CO_2, 10% O_2, 80% N_2 ("10/10", graph 1C). Irradiation doses are 0.0 (circle), 0.3 (triangle) or 0.6 kGy (square). Error bars indicate standard error, $n = 9$.

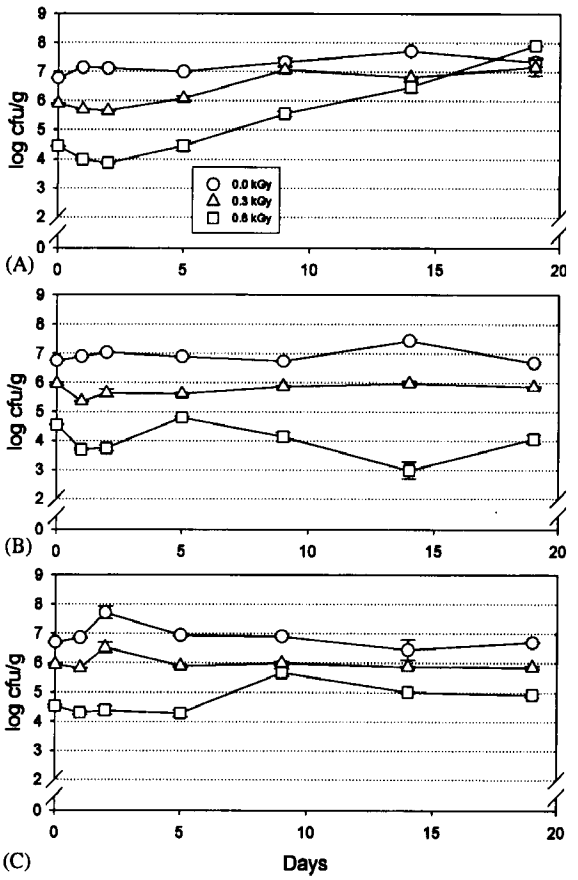

Fig. 2. Total microbial populations from packaged, irradiated endive, plated on tryptic soy agar. Packaging atmospheres are air ("Air-0", graph 2A), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 ("5/5", graph 2B) or 10% CO_2, 10% O_2, 80% N_2 ("10/10", graph 2C). Irradiation doses are 0.0 (circle), 0.3 (triangle) or 0.6 kGy (square). Error bars indicate standard error, $n = 9$.

and C, respectively) and total microflora (Fig. 2B and C, respectively) tended not to increase during storage, and were not significantly ($P<0.05$) different than the initial levels. On non-irradiated samples, the populations observed during storage tended to be more variable on 5/5 than in either Air-0 or 10/10. Also in contrast to the regrowth observed in Air-0 samples, the *L. monocytogenes* and total microbial populations on the irradiated 5/5 and 10/10 samples at the final sampling times (day 19) were either significantly ($P<0.05$) lower than or not significantly different from the initial levels.

Irradiation reduced the levels of *L. monocytogenes* and total microflora in each of the three atmospheres examined. A dose of 0.6 kGy resulted in significant ($P<0.05$) reductions of 3.09 (Fig. 1A), 2.41 (Fig. 1B) and 2.53 (Fig. 1C) $\log_{10}$ cfu/g of *L. monocytogenes* in Air-0, 5/5 and 10/10, respectively. The same dose significantly ($P<0.05$) reduced the total microflora by 2.34 (Fig. 2A), 2.20 (Fig. 2B) and 2.26 (Fig. 2C) $\log_{10}$ cfu/g in Air-0, 5/5 and 10/10, respectively. On irradiated Air-0 samples (Fig. 1A), at both 0.3 and 0.6 kGy, *L. monocytogenes* and the total microflora regrew from the initial reductions until the population was equal to or significantly greater ($P<0.05$) than the non-irradiated samples by the last sampling time (19 days). These results support earlier reports of initial reduction, followed by the regrowth in storage of *L. monocytogenes* on irradiated vegetables and meats stored in aerobic packaging. In those studies, *L. monocytogenes*-inoculated celery (Prakash et al., 2000a, b), endive (Niemira et al., 2003), frankfurters (Sommers and Fan, 2003) and beef bologna (Sommers et al., 2003) were treated with doses resulting in initial reductions of approximately 2 $\log_{10}$ cfu/g, comparable to those obtained in the present work. The pattern of regrowth following a low radiation dose is consistent,

despite the fact that the D_{10} value, i.e. the radiation dose required to achieve a $1 \log_{10}$ reduction, for *L. monocytogenes* is typically higher on meats than on vegetables.

In contrast to the results obtained in the Air-0 packages, *L. monocytogenes* did not regrow following irradiation on endive packaged in 5/5 (Fig. 1B) or 10/10 (Fig. 1C). Recoverable counts from the latter sampling dates under both gas regimes were either significantly ($P<0.05$) lower than, or not different from, the levels seen immediately post-irradiation (day 0). Total microbiological counts showed a similar pattern of persistent post-irradiation suppression under 5/5 (Fig. 2B) and 10/10 (Fig. 2C) packaging. These results demonstrate that the antimicrobial effects of irradiation can be made to persist during refrigerated storage by altering the headspace gas composition.

The hyperbolic model was adequate to describe the regrowth after treatment with 0.6 kGy of *L. monocytogenes* ($R^2 = 0.92$) and total microbial counts ($R^2 = 0.90$) in Air-0 packages. However, the hyperbolic model was inadequate to describe the behavior of microorganisms in other treatment combinations.

3.2. Headspace gas

In each of the atmospheres used, the percentage O_2 decreased and that of CO_2 increased (Fig. 3A). For Air-0 samples, CO_2 increased from less than 1% at the start of the study to 18–23% at the final sampling time, with no significant difference among the three radiation levels. Levels of O_2 declined from initial measurements of 18–19% to final levels of 1–6%, also with no significant difference resulting from irradiation. For samples packaged in both the 5/5 (Fig. 3B) and 10/10 (Fig. 3C) atmospheres, O_2 levels dropped to below detectable levels (less than 1%) by 5 days post-packaging for all three radiation doses. Levels of CO_2 rose during storage in samples packed in both 5/5 and 10/10, but tended to be more variable in 5/5.

Elevated levels of CO_2 can have a bacteriostatic or bactericidal effect on spoilage pathogens (Yuan, 2003). By day 5, CO_2 levels in the Air-0 samples rose to 8–13% and O_2 levels fell to 4–8% (Fig 3A), yet the irradiated samples were still subject to a regrowth of *L. monocytogenes* and total microflora. However, the combination of elevated CO_2 and very low O_2 levels in the 5/5 and 10/10 samples during the course of storage prevented the regrowth of bacteria on the irradiated samples in both atmospheres.

3.3. Sensory properties

3.3.1. Texture

There were no significant ($P<0.05$) differences in texture (maximum shear force) resulting from irradiation in any atmosphere, at any given sampling date (Table 1). Similarly, there were no significant differences among the atmospheres at any given sampling date (Table 1). In Air-0 samples, there was no significant ($P<0.05$) difference between the day 1 and day 8 samples at any of the three doses. Samples packaged in 5/5 and 10/10 generally had a significant loss of texture by day 8. However, extended time in storage caused an essentially similar pattern of loss of texture for all gas/dose combinations, in that samples which were to have been evaluated at day 14 were degraded to the point that meaningful measurements of shear force could not be taken (Table 1). As commercially packaged leafy salad vegetables have a salable shelf life of 12–14 days (Garrett, 2002), the loss of texture at the final sampling time in this study is not unexpected.

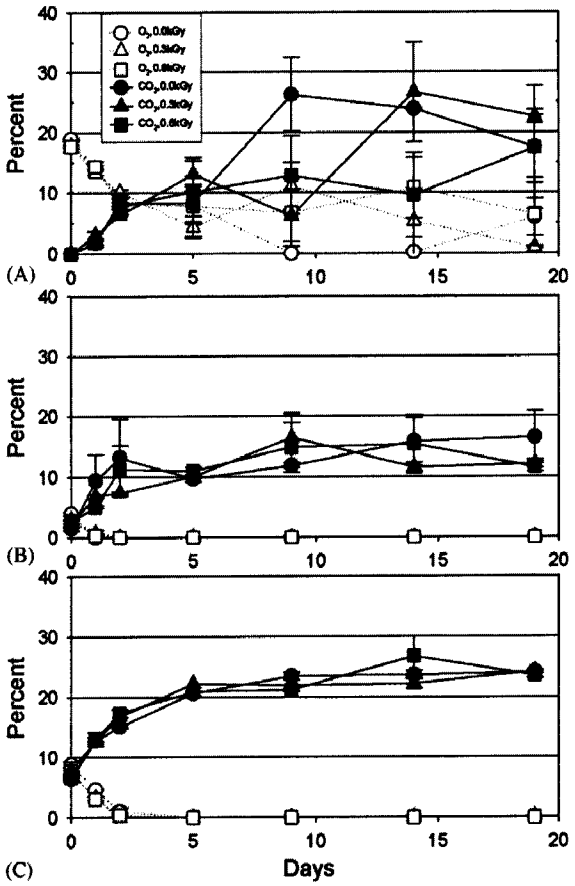

Fig. 3. Concentration of O_2 (white) and CO_2 (black) in packaged, irradiated endive. Packaging atmospheres are air ("Air-0", graph 3A), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 ("5/5", graph 3B) or 10% CO_2, 10% O_2, 80% N_2 ("10/10", graph 3C). Irradiation doses are 0.0 (circle), 0.3 (triangle) or 0.6 kGy (square). Error bars indicate standard error, $n = 3$.

Table 1
Texture (maximum shear force in g) of irradiated endive stored at 4°C for 14 days[a]

Day	Gas	0.0 kGy	0.3 kGy	0.6 kGy
1	Air-0	25357±1170abx	24744±1602abx	24997±1622abx
8	Air-0	20398±1398bcx	23208±1667bcx	19852±1356bcx
14	Air-0	N/A	N/A	N/A
1	5/5	26741±1349abx	28735±1083ax	26055±1252ax
8	5/5	19535±2119bcx	18732±1306cx	18654±1686cx
14	5/5	N/A	N/A	N/A
1	10/10	24204±1217abx	26322±936abx	28383±952ax
8	10/10	17422±1516cx	18811±1171cx	22926±1648abcx
14	10/10	N/A	N/A	N/A

N/A: data not available due to sample degradation.
[a]Within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different ($P<0.05$). Within each row, means indicated by the same letter (x through z) are not significantly different ($P<0.05$) (±SE).

Table 2
Color values (greenness) of irradiated endive stored at 4°C for 14 days[a]

Day	Gas	0.0 kGy	0.3 kGy	0.6 kGy
1	Air-0	−8.9±0.16dx	−8.6±0.16ex	−8.6±0.17cx
8	Air-0	−7.8±0.27cdx	−8.3±0.12dex	−7.6±0.39bcx
14	Air-0	−5.0±0.40bxy	−6.5±0.38abcx	−5.6±0.67aby
1	5/5	−8.5±0.14cdx	−8.3±0.20cdex	−8.8±0.12cx
8	5/5	−7.4±0.27cx	−6.9±0.24abcdxy	−6.2±0.27aby
14	5/5	−3.9±0.20ax	−4.3±0.24ax	−3.9±0.39ax
1	10/10	−8.7±0.22dx	−8.6±0.13ex	−8.6±0.16cx
8	10/10	−8.2±0.21cdx	−7.4±0.36bcdex	−7.7±0.24bcx
14	10/10	−5.7±0.30bx	−5.3±0.21abx	−4.8±0.37ax

[a]Within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different ($P<0.05$). Within each row, means indicated by the same letter (x through z) are not significantly different ($P<0.05$) (±SE).

Table 3
Color values (brightness) of irradiated endive stored at 4°C for 14 days[a]

Day	Gas	0.0 kGy	0.3 kGy	0.6 kGy
1	Air-0	36.1±0.96abx	34.5±1.10abx	35.9±1.02abx
8	Air-0	35.0±0.79abcx	36.1±0.80abx	37.3±1.82ax
14	Air-0	28.7±1.50dx	33.5±1.41aby	35.4±0.79aby
1	5/5	36.3±0.63abx	36.0±0.92abx	37.6±1.09ax
8	5/5	34.0±0.97bcx	33.2±0.76abx	33.4±1.09abcx
14	5/5	27.9±1.11dx	31.6±1.44bx	30.0±1.45cx
1	10/10	38.5±0.80ax	37.5±0.82ax	37.3±0.85ax
8	10/10	35.0±0.89abcx	34.0±1.15abx	36.3±1.18abx
14	10/10	31.0±0.79cdx	31.8±0.99bx	31.1±1.11bcx

[a]Within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different ($P<0.05$). Within each row, means indicated by the same letter (x through z) are not significantly different ($P<0.05$) (±SE).

3.3.2. Color

All gas/dose combinations were subject to significant ($P<0.05$) loss of greenness (i.e higher "a" values) during storage (Table 2). Neither radiation dose nor the packaging atmosphere employed had a consistent effect on "a" values.

In the non-irradiated samples in each of the three atmospheres, day 14 samples were significantly darker (i.e. lower "L" values) than the day 1 samples (Table 3). In materials which had received 0.3 kGy, this pattern was repeated for 10/10 samples, but the loss did not rise to the level of significance for Air-0 and 5/5 samples. Following 0.6 kGy, there was significant darkening for 5/5 and 10/10 samples at day 14, but not for Air-0 samples.

A somewhat similar pattern was observed with regard to loss of yellowness (i.e. lower "b" values) (Table 4). In the non-irradiated samples in each of the three atmospheres, day 14 samples were significantly ($P<0.05$) less yellow than the day 1 samples. Following 0.3 kGy, 5/5 samples showed significant loss of yellowness by day 14, but Air-0 and 10/10 samples did not. Following 0.6 kGy, there was a significant loss of yellowness for 5/5 and 10/10 samples by day 14, but not for Air-0. It should be noted that for each of these color assessments, the results obtained at the final sampling time, day 14, are of limited value, as the loss of texture at this time would render the product essentially unsalable.

This study shows that the reduction in *L. monocytogenes* in the 5/5 and 10/10 samples following low-dose irradiation can be made to persist during storage by forcing the O_2 content of the atmosphere to very low levels. However, the resultant loss of texture and color resulting from the anaerobic conditions within the sample bags specific atmospheres used herein highlight the equally important need for providing the leaves with

Table 4
Color values (yellowness) of irradiated endive stored at 4°C for 14 days[a]

Day	Gas	0.0 kGy	0.3 kGy	0.6 kGy
1	Air-0	23.3±0.45ax	22.2±0.41abcx	21.8±0.51abx
8	Air-0	22.1±0.40abx	22.9±0.40abx	22.3±0.63ax
14	Air-0	20.3±0.85bcx	23.0±0.74ay	22.5±0.39ay
1	5/5	22.4±0.55abx	22.2±0.31abxc	23.2±0.60ax
8	5/5	22.6±0.47abx	22.4±0.31abx	22.2±0.38ax
14	5/5	18.6±0.52cx	19.8±0.78cx	19.2±0.46cx
1	10/10	23.5±0.47axy	21.9±0.43abcx	22.4±0.48ay
8	10/10	23.4±0.51ax	21.9±0.78abcx	21.7±0.59abx
14	10/10	20.3±0.56bcx	20.5±0.55bcx	19.6±0.60bcx

[a] Within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different ($P<0.05$). Within each row, means indicated by the same letter (x through z) are not significantly different ($P<0.05$) (±SE).

an opportunity for gas exchange. The texture and color of the material in this study further demonstrates that vegetables hold their quality better in an environment which provides some level of O_2, even as part of a high-CO_2, low-O_2 atmosphere. The undesirable post-irradiation regrowth of *L. monocytogenes* observed in this atmosphere suggests, however, that finer control of the product microenvironment is required. It has been well documented that packaged vegetables conduct respiration (consume O_2 and generate CO_2) during storage, transpire water, and that a fully anaerobic gas environment in storage can be detrimental to vegetable quality (Fan et al., 2003; Yuan, 2003). Because of this, commercial MAP applications typically rely on packaging which is oxygen permeable. The oxygen transmission rate of commercially used plastics varies widely, and range from very low to excess of 200 cc/h/m^2 (Al-Ati and Hotchkiss, 2002). Semi-permeable film bags can, by adjusting the gas transmission rate, establish a gas mixture suitable to maintain product quality; a challenge is balancing the preservation of quality while preventing the regrowth of *L. monocytogenes* following low-dose irradiation.

The use of additional control factors such as chemical sanitizers, antimicrobial agents or other interventions applied in conjunction with MAP and irradiation is an important area of future research. The consistent pattern of regrowth following low irradiation doses, even on very dissimilar products such as vegetables and meats, indicates that where irradiation is used as an intervention in products intended for extended refrigerated storage, a dose greater than three D_{10} units should be used. As the radiation sensitivity of pathogens can vary based on the food product substrate, the actual dose applied to the product will depend on validation with the product of interest. Therefore, a hurdle system which uses irradiation as one step in a multi-intervention process for the sanitization of vegetables or meats must be calibrated and validated to obtain lasting benefit from the irradiation component of the overall process.

4. Conclusion

Irradiation significantly reduced initial levels of *L. monocytogenes* and total microflora under each of the three atmospheres examined. During storage, *L. monocytogenes* and total microflora regrew on the irradiated Air-0 samples until the levels were equal or greater than the non-irradiated samples by Day 19. In contrast, the *L. monocytogenes* and total microbial populations on the irradiated 5/5 and 10/10 samples remained at or close to the initial reduced levels. In each of the three atmospheres, O_2 declined and CO_2 increased, irrespective of radiation dose. Irradiation had no immediate significant effect on texture or color. Irradiated leaf material in Air-0 tended to retain color attributes during storage better than non-irradiated; color retention was more variable under 5/5 and 10/10 packaging. After 8 days, maximum shear force relative to the initial level was significantly reduced in 5/5 at all radiation doses, not significantly changed in Air-0, and dose-dependent in 10/10. By 14 days, the texture of all samples had degraded significantly. These results indicate that irradiation and MAP can be combined to reduce levels of *L. monocytogenes* and prevent its regrowth during storage, thereby serving to protect the consumer, but that the process must be further optimized to preserve product quality.

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References

Al-Ati, T., Hotchkiss, J.H., 2002. Application of packaging and modified atmosphere to fresh-cut fruits and vegetables. In: Lamikanra, O. (Ed.), Fresh-Cut Fruits and Vegetables, Science, Technology and Market. CRC Press, Boca Raton, FL, pp. 305–338.

Beuchat, L.R., 1996. *Listeria monocytogenes*: incidence in vegetables. Food Control 7, 223–228.

Fan, X., Toivonen, P.M.A., Rajkowski, K.T., Sokorai, K.J.B., 2003. Warm water treatment in combination with modified atmosphere packaging reduces undesirable effects of irradiation on the quality of fresh-cut iceberg lettuce. J. Agric. Food Chem. 51, 1231–1236.

Foley, D.M., Dufour, A., Rodriguez, L., Caporaso, F., Prakash, A., 2002. Reduction of *E. coli* O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. Radiat. Phys. Chem. 63, 391–396.

Garrett, E.H., 2002. Fresh-cut produce: tracks and trends. In: Lamikanra, O. (Ed.), Fresh-Cut Fruits and Vegetables, Science, Technology and Market. CRC Press, Boca Raton, FL, pp. 1–10.

Gombas, D.E., Chen, Y., Clavero, R.S., Scott, V.N., 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Prot. 66 (4), 559–569.

Hagenmaier, R.D., Baker, R.A., 1997. Low-dose irradiation of cut iceberg lettuce in modified atmosphere packaging. J. Agric. Food. Chem. 45, 2864–2868.

Haldane, D., 2003. Outbreak of *E. coli* appears contained: in San Diego and Orange counties, 28 cases are reported—lettuce source is traced. Los Angeles Times, October 10, 2003.

Horby, P.W., O'Brien, S.J., Adak, G.K., Graham, C., Hawker, J.I., Hunter, P., Lane, C., Lawson, A.J., Mitchell, R.T., Reacher, M.H., Threlfall, E.J., Ward, L.R., 2003. A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. Epidemiol. Infect. 130, 169–178.

Niemira, B.A., 2003. Irradiation of fresh and minimally processed fruits, vegetables and juices. In: Novak, J.S., Sapers, G.M., Juneja, V.K. (Eds.), The Microbial Safety of Minimally Processed Foods. CRC Press, Boca Raton, FL, pp. 279–300.

Niemira, B.A., Sommers, C.H., Fan, X., 2002. Suspending lettuce type influences recoverability and radiation sensitivity of *E. coli* O157:H7. J. Food Prot. 65 (9), 1388–1393.

Niemira, B.A., Fan, X., Sokorai, K.J.B., Sommers, C.H., 2003. Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and *L. innocua* ATCC 51742 inoculated on endive (*Cichorium endiva*). J. Food Prot. 66, 993–998.

Prakash, A., Guner, A.R., Caporaso, F., Foley, D.M., 2000a. Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut Romaine lettuce packaged under modified atmosphere. J. Food Sci. 65 (3), 549–553.

Prakash, A., Inthajak, P., Huibregtse, H., Caporaso, F., Foley, D.M., 2000b. Effects of low-dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. J. Food Sci. 65 (6), 1070–1075.

Sommers, C.H., Fan, X., 2003. Irradiation (gamma) of fine emulsion sausage that contained sodium diacetate. J. Food Prot. 66, 819–824.

Sommers, C.H., Fan, X., Niemira, B.A., Sokorai, K., 2003. Radiation (Gamma) resistance and post-irradiation growth of *Listeria monoctytogenes* suspended in beef bologna that contained sodium diacetate and potassium lactate. J. Food Prot. 66 (11), 2051–2056.

Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Madden, J., Wachsmuth, K., 1997. Microbial hazards and emerging issues associated with produce: a preliminary report to the national advisory committee on microbiological criteria for foods. J. Food Prot. 60 (11), 1400–1408.

Yuan, J.T.C., 2003. Modified atmosphere packaging for shelf-life extension. In: Novak, J.S., Sapers, G.M., Juneja, V.K. (Eds.), The Microbial Safety of Minimally Processed Foods. CRC Press, Boca Raton, FL, pp. 205–219.